Gingival inflammation, increased periodontal pocket depth and elevated interleukin-6 in gingival crevicular fluid of depressed women on long-term sick leave

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Background and Objective: The aim of this work was to investigate periodontal status, in relation to inflammatory markers and cortisol, in the gingival crevicular fluid and saliva of a homogenous group of women on long-term sick leave for job-stress related depression in comparison to nondepressed women.

Material and Methods: The participants comprised 20 women with depression (DSM-IV) (mean age 48.5 $\pm$ 6.9 years) and 29 healthy controls (mean age 54.5 $\pm$ 2.9 years). Clinical examination was performed. Gingival crevicular fluid was collected by an intracrevicular washing technique. Interleukin-1β, interleukin-6, matrix metalloproteinase (MMP)-8 and MMP-9 were determined with enzyme-linked immunosorbent assay and cortisol was determined by using a radioimmunoassay. One-way analysis of covariance was used as the statistical method.

Results: The depressed patients had significantly higher gingival inflammation ($p < 0.001$), and deeper pockets ($p < 0.003$), than the healthy controls, after adjusting for age and smoking. The levels of interleukin-6 in the gingival crevicular fluid were significantly higher in the patients than in the controls: 3.84 $\pm$ 1.58 pg per site and 0.79 $\pm$ 1.83 pg per site, respectively, $p < 0.003$. There were no significant differences in the levels of interleukin-1β, MMP-8 and MMP-9. The patients had lower cortisol values in gingival crevicular fluid than the controls, whereas the levels of cortisol in saliva were similar in both groups.

Conclusion: Women on long-term sick-leave for depression had more severe periodontitis and higher concentrations of interleukin-6 in gingival crevicular fluid than healthy controls. An alteration of the immune system in these patients might be interpreted as reflecting the consequences of long-term stress exposure and might contribute to worse periodontal conditions in these particular patients.

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An association between psychosocial stress and gingival inflammation has been demonstrated in a number of recent studies (1–4). In patients with severe periodontitis, the following background factors have been implicated: psychosocial stress (such as financial insecurity and poor coping strategies) (5); traumatic life events (e.g. loss of a spouse) (6); passive stress-coping strategies (7); and high trait anxiety (8). In periodontitis patients with clinical depression, periodontal treatment outcomes are less favourable than in those without depression (9).

Acute psychological stress has been associated with enhanced serum levels of interleukin-6 (10). Increased levels of serum interleukin-1β and interleukin-6 have also been reported in patients with major depression and post-traumatic stress disorder (11,12). Stress has also been related to increased levels of interleukin-1β and interleukin-6 in the gingival crevicular fluid of patients with periodontitis (13,14).

During the last decade, psychological factors, such as stress and depression, have been identified as contributing to the progression of periodontal disease and to changes in immune mechanisms (15). Although the mechanisms of interaction have not yet been clarified, it has been suggested that stress-related alterations in the immune response may facilitate colonization by pathogenic bacteria, which in turn may lead to the destruction of periodontal tissue.

The psychosocial stress reaction includes activation of the hypothalamus–pituitary–adrenal cortex axis, with release of corticotrophin-releasing hormone from the hypothalamus and of glucocorticoids (including cortisol) from the adrenal cortex (16). A marker commonly used to study the function of the hypothalamus–pituitary–adrenal cortex axis during stress is the salivary concentration of cortisol (17). Studies of patients with stress-related psychiatric conditions have, however, yielded contradictory results: Melamed *et al.* (18) and Grossi *et al.* (19) reported increased concentrations, whereas decreased levels were reported by Pruessner *et al.* (20) and Rohleder *et al.* (21).

Recently, a comparative study of women with stress-related depression, and of healthy controls, disclosed that the depressed subjects had elevated levels of cortisol in gingival crevicular fluid and a higher incidence of gingivitis (14).

The aim of the present study was to investigate periodontal status, in relation to inflammatory markers and cortisol, in the gingival crevicular fluid and saliva of a more homogenous group of women on long-term sick leave for job stress-related depression in comparison to nondepressed women.

Material and methods

Patients

The patients were recruited from a study of women on long-term sick leave for job stress-related depression. For that study, patients were recruited from lists of subjects on long-term sick leave, maintained by a large Swedish insurance company (AFA). Two-hundred and ninety women, between 40 and 50 years of age, employed in the public healthcare or social service sector, who had been on sick leave for 3–8 mo with a psychiatric disorder (excluding psychosis or substance abuse) were approached by letter. Fifty-five women declined to be contacted by telephone and 37 could not be reached. Of the remaining 198 subjects, all were interviewed by telephone, and 44 women who met the preliminary inclusion criteria participated in a detailed psychiatric examination, including a diagnostic interview by the Structured Clinical Interview (22,23), which yields a diagnosis according to the *Diagnostic and Statistical Manual for Mental Disorders, Fourth Edition* (DSM-IV) (24). Thirty-one subjects had been accepted for this study, which involved hormonal challenge tests and magnetic resonance tomographic scans; for further details see Rydmark *et al.* (25). For inclusion, the subject must have fulfilled criteria for either a major depression or a maladaptive stress disorder with depressed mood, thought to be induced by severe job stress for longer than 6 mo. Patients seen between November 2002 and November 2003 were invited to

participate in the present study. In all, 31 women were invited to participate and 22 accepted after receiving information about the aims and methods of the study. Two prospective patients taking hormonal supplements (estrogens) were excluded.

Twenty patients (mean age 48.5 $\pm$ 6.9 years) were finally enrolled in the periodontal study. Eighteen had a diagnosis of current major depression, or a major depression in partial remission, and two had a maladaptive stress reaction. Nine of the patients were on antidepressants (serotonin uptake inhibitors in all cases), and a subgroup analysis was performed for the 11 patients who were not taking antidepressants. No patients were pregnant or used contraceptive pills, and none had received antibiotics during the previous 3 mo.

Controls

The subjects in the control group were recruited from a random population sample, selected and drawn from the registry file of all inhabitants of the Stockholm area (26). The initial sample consisted of 3273 people, born on the 20th day of any month from 1945 to 1954. A subsample of 45 women was randomly selected from this group. The women were contacted by telephone, and the aims and procedures of the study were explained to them. Four women declined to participate in the study, and eight women could not be reached by telephone, either because they had unlisted numbers or because they did not answer after repeated attempts to contact them. Three women had moved from Stockholm and it was impossible for them to attend the study. Thirty women accepted the invitation to participate in the study and they were sent a letter describing its aims and procedures. The exclusion criteria were: self-reported psychiatric disorder; use of psychotropic medication; pregnancy; or use of oral contraceptives or estrogens. One subject was excluded because of the use of estrogens. The subjects were in good general health, as assessed by a health questionnaire. None had received antibiotics during the previous

3 mo. The final control group comprised 29 subjects (mean age 54.5 ± 2.9 years).

Ethics

This study was approved by the Ethics Committee at Huddinge University Hospital (Huddinge, Sweden). The subjects gave their informed consent to participate in the study.

Saliva collection procedures

To avoid contamination of the oral cavity as a result of food intake or smoking, the subjects were instructed not to eat, drink or smoke within 1 h of sampling. To minimize the risk of blood contamination, toothbrushing was not allowed during the 60 min preceding saliva collection. The subjects were instructed to swallow before saliva sampling and then to spit all saliva produced during a 5-min period into a test tube. All samples were collected between 08:30 h and 09:00 h. The samples were immediately centrifuged (8000 g, 15 min, 4°C) and the supernatants were then frozen at −70°C, pending analysis.

Clinical examination

The clinical examination was conducted after saliva sampling, and included assessment of the presence of dental plaque on lingual and buccal surfaces (1 = plaque, 0 = no plaque), gingival index (27), and the number of remaining teeth, excluding third molars. Probing pocket depth and clinical attachment level were measured to the nearest millimeter, using a standard probe (Hu-Friedy, Chicago, IL, USA) graded at 2 mm intervals and with a tip diameter of 0.5 mm. All teeth were probed at six sites: mesio-buccal; mesio-lingual; mid-buccal; disto-buccal; disto-lingual; and mid-lingual. Clinical attachment level was measured with a probe from the cemento–enamel junction. Bleeding on probing was assessed by probing intracrevicularly, using a probe with a tip diameter of 0.5 mm (Hu-Friedy). Bleeding within 60 s was recorded as 'bleeding on probing'. One examiner (AJ) performed all measurements.

Gingival crevicular fluid sampling

The most inflamed site in each quadrant was selected for sampling of gingival crevicular fluid, which was then analyzed for levels of interleukin-1β, interleukin-6, matrix metalloproteinase (MMP)-8, MMP-9 and cortisol. The sites to be sampled were isolated with cotton rolls, gently air dried and the supragingival plaque carefully removed. The ejection needle of the instrument was then gently inserted into the crevice to a level 1 mm below the gingival margin. Gingival crevicular fluid was collected using the intracrevicular washing technique (28). The samples were immediately centrifuged (8000 g) for 15 min at 4°C and the supernatants were then frozen at −70°C pending analysis.

Assays

Interleukin-1β and interleukin-6 — Interleukin-1β and interleukin-6 were measured using enzyme-linked immunosorbent assay (ELISA) Quantikine HS Immunoassay Kits (R & D Systems Europe Ltd, Oxon, UK), according to the manufacturer's instruction manual. The levels of interleukin-1β and interleukin-6 were determined as the total amount per site (pg per site).

MMP-8 and MMP-9 — Gingival crevicular fluid supernatants were assayed for MMP-8 and MMP-9 (both free and complexed) using an ELISA (R & D Systems Europe Ltd), according to the manufacturer's instruction manual. The levels of MMP-8 and MMP-9 were determined as the total amount per site (ng per site).

Cortisol in gingival crevicular fluid and saliva — Cortisol was measured using a sensitive radioimmunoassay (Orion Diagnostica AB, Espoo, Finland), according to the manufacturer's instructions. The levels of cortisol were determined as total amounts per site (nmol/L).

Statistical analysis

The data analyses were performed using the statistical packages of STATVIEW 5.0.1 (SAS Institute Inc., Cary, NC, USA). When comparing dental observations and inflammatory markers and cortisol for patients and controls, an analysis of covariance was used to remove the influence of age and smoking. To correct the *p*-values for multiple comparisons, Bonferroni's method was used. The Fisher's exact *t*-test was used to determine the significance of detectable and nondetectable cortisol values in gingival crevicular fluid between the groups.

Results

Descriptive characteristics of the patients and controls are shown in Table 1, and the clinical data (means and standard deviations) are shown in Table 2. After adjusting for age and smoking, significant differences were found between patients and controls with respect to gingival inflammation ($p < 0.000$) and probing pocket depth ($p < 0.003$) (Table 2). There were no significant differences in any of the clinical parameters between patients on antidepressants and those not taking antidepressants.

The level of interleukin-6 was significantly higher ($p < 0.001$) in patients than in controls (Table 3). Interleukin-6 was detected in all patient samples

Table 1. Descriptive characteristics of the depressed patients and controls

	Patients ($n = 20$)	Controls ($n = 29$)
Level of education		
1–9 years	5	4
10–12 years	5	11
> 12 years	10	14
Family situation		
Married	15	21
Divorced	2	3
Single	3	5
Current smokers	8	8
Smoked years	25.5	30.6
Cigarettes per day	12.3	13.2
Former smokers	3	15
Quit smoking/years	14.5	17.2
Cigarettes per day	15.0	11.8
Prescribed antidepressants	9	0

The values shown represent the number of patients.

Table 2. Dental plaque, gingival inflammation, bleeding on probing, pocket depth, clinical attachment level, and number of teeth in patients and controls, after controlling for age and smoking, by analysis of covariance (ANCOVA)

	Patients ($n = 20$)		Controls ($n = 29$)	
	Mean $\pm$ SD[a]	Mean $\pm$ SD	F_{DF}[b]	*p*-value
Dental plaque[c]	0.22 $\pm$ 0.13	0.10 $\pm$ 0.10	4.62 $_{1,45}$	0.037
Gingival inflammation	1.59 $\pm$ 0.34	0.89 $\pm$ 0.35	32.36 $_{1,45}$	0.000[d]
Bleeding on probing (%)	60.98 $\pm$ 25.29	35.86 $\pm$ 23.98	7.76 $_{1,45}$	0.008
Probing depth (mm)	3.06 $\pm$ 0.29	2.57 $\pm$ 0.50	9.73 $_{1,45}$	0.003[d]
Clinical attachment level (mm)	2.34 $\pm$ 0.19	2.39 $\pm$ 0.60	0.51 $_{1,45}$	0.478
Number of teeth	27.10 $\pm$ 1.29	26.51 $\pm$ 1.32	2.33 $_{1,45}$	0.1335

[a]Standard deviation of the mean.
[b]DF, degrees of freedom.
[c]Buccal/lingual sites.
[d]Multiple comparisons, according to Bonferroni's method.
n = number of subjects.

Table 3. Interleukin-1β, interleukin-6, matrix metalloproteinases-8 and -9, cortisol in gingival crevicular fluid, and cortisol in saliva, in patients and controls, controlling for age and smoking by analysis of covariance

		Patients ($n = 20$)		Controls ($n = 29$)	
	Unit	Mean $\pm$ SD[a]	Mean $\pm$ SD	F_{DF}[b]	*p*-value
Interleukin-1β	pg per site	32.07 $\pm$ 14.92	31.65 $\pm$ 20.61	0.82 $_{1,45}$	0.370
Interleukin-6	pg per site	3.84 $\pm$ 1.58	0.79 $\pm$ 1.83	22.91 $_{1,45}$	0.000[c]
Matrix metalloproteinase-8	ng per site	12.49 $\pm$ 9.90	11.98 $\pm$ 5.56	0.10 $_{1,45}$	0.920
Matrix metalloproteinase-9	ng per site	28.26 $\pm$ 20.14	30.56 $\pm$ 18.49	0.15 $_{1,45}$	0.704
Cortisol in gingival crevicular fluid[d]	nmol/L	0.17 $\pm$ 0.33	0.30 $\pm$ 0.25	0.62 $_{1,45}$	0.436
Cortisol in saliva	nmol/L	12.20 $\pm$ 4.79	10.50 $\pm$ 5.07	0.01 $_{1,45}$	0.907

[a]Standard deviation of the mean.
[b]DF, degrees of freedom.
[c]Multiple comparisons, according to Bonferroni's method.
[d]Gingival crevicular fluid.
n = number of subjects.

and in 30% of samples from the controls. The levels of interleukin-1β, MMP-8 and MMP-9 did not differ significantly between patients and controls (Table 3). The levels of cortisol in saliva were also similar in patients and controls (Table 3). In contrast, the mean level of cortisol in the gingival crevicular fluid was lower in the patients than in the controls and was detected in only 27% of samples from patients, but in 96.5% of samples from controls ($p < 0.001$; Fisher's exact *t*-test). Correlation between the percentage of gingival inflammation (gingival index) and plaque (plaque index) was determined in 20 depressed patients ($r^2 = 0.0297$, $p = 0.4679$) and 29 controls ($r^2 = 0.3970$, $p = 0.0002$) (Fig. 1).

Analysis of the effects of antidepressant medication failed to disclose any difference in any of the biochemical markers between patients who took antidepressants and those who did not.

Discussion

The present study disclosed that women on long-term sick leave for job stress-related depression tend to have more gingival inflammation and deeper periodontal pockets than healthy controls. The increased level of gingival inflammation may be explained by behavioral changes in the depressed patients, such as inappropriate coping styles, neglect of oral hygiene, or changes in eating habits, which may be harmful to periodontal health and can lead to aggravated inflammation and an increased susceptibility to periodontitis. To reduce the effect of behavioural changes as a confounder for increased levels of gingivitis in depressed patients, a separate analysis was carried out on two groups of subjects with the same levels of plaque. In the control group, as expected, the levels of gingivitis increased following an increase of plaque, whereas in the depressed patients the gingivitis level was increased also in patients with very low levels of plaque, thus indicating an influence of depression on gingivitis, regardless of the plaque levels. This might be explained by a change in hypothalamus–pituitary–adrenal cortex axis activity, leading to an alteration in the host response that may result in a lowered host resistance to periodontitis-related bacteria (29,30). Similar findings were reported by Klages *et al.* (2), who found that patients with more severe depression symptoms also had more sulcus bleeding in the gingiva. In a previous investigation from our group (14), we found that patients with stress-related exhaustion and depression had more gingival inflammation than healthy controls. Depression may also cause salivary flow rates to decrease, thus making the individual more susceptible to oral diseases (15).

The depressed patients in the present study had deeper periodontal pockets, which may increase the risk for development or for the progression of periodontal disease. Other investigators have evaluated the effect of occupational stressors on periodontal health status, and report more gingivitis and deeper pocket depth in such patients (31,32). This is in line with the fact that our patients had developed their illness probably as a consequence of work stress.

In this study we also assessed the gingival crevicular fluid levels of two inflammatory related cytokines: interleukin-1β and interleukin-6. The level of interleukin-6 was significantly higher in the patients than in the controls, whereas the level of interleukin-1β was similar in both groups. These increased levels of interleukin-6 in the present study, suggest that depression may
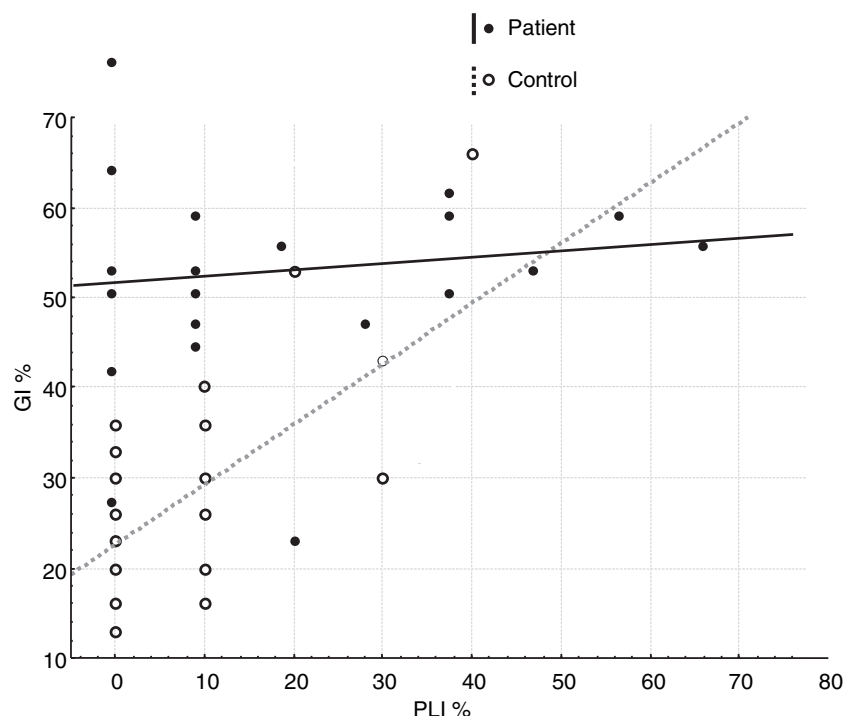
Fig. 1. Correlation between percentage of gingival inflammation and plaque in depressed patients ($r^2 = 0.0297$, $p = 0.4679$) and 29 controls ($r^2 = 0.3970$. $p = 0.0002$).

stimulate the production of interleukin-6. Higher levels of interleukin-6 have previously been observed in patients with different psychiatric disorders (14,33,34). It has been proposed that depressed patients exhibit alterations in both the cellular (35,36) and humoral (37) immune responses, which may impair immunological defence mechanisms and promote accumulation of periodontopathogens that exacerbate periodontal diseases. However, whether the increase of interleukin-6 levels is one of the causes of the periodontitis, or is increased because of the presence of periodontitis, cannot be established in this study; for this possible cause–effect relationship, an intervention study is needed.

The lack of change in interleukin-1β level in the patient group was also found in a previous study (14). In a review, Chrousos (16), stated that systemic interleukin-6 concentrations increase during stress unrelated to inflammation, and that interleukin-6 inhibits the secretion of interleukin-1. This may explain why increased concentrations of interleukin-6 were found in the absence of changes in interleukin-1β.

Somewhat surprisingly, the gingival crevicular fluid levels of cortisol were lower in the patients than in the controls, whereas the salivary levels of cortisol were similar in the two groups. The only other study of gingival crevicular fluid cortisol that we have been able to retrieve from the literature – that of Axtelius *et al.* (38) – used a different sampling technique and found higher cortisol concentrations in gingival crevicular fluid from inflamed sites. A previous study from our group found higher gingival crevicular fluid cortisol levels in patients with stress-related depression (14), who also had more gingival inflammation. As in that study, no differences in salivary cortisol were found between patients and controls. Different markers of the hypothalamus–pituitary–adrenal cortex axis function in depressed patients usually suggest increased activation (19), whereas in atypical depression (39), and in post-traumatic stress disorder (21), a lowered reactivity of the hypothalamus–pituitary–adrenal cortex axis response has been found. The reason for this discrepancy is not easily explained, because our two patients

groups are clinically similar. However, the group in the present study was more homogenous as prolonged work stress was an inclusion criterion. Interestingly, the patients in the present study also participated in a challenge study of hypothalamus–pituitary–adrenal cortex axis function, where the reaction of both cortisol and adrenocorticotropin hormone to corticotrophin-releasing hormone/dexamethasone challenge was attenuated (25).

In addition, the sampling time might also influence the results obtained. All samples in the present study were taken at the same time in the morning, in all cases, which is preferable, but a limitation of our study was that saliva analysis was based on a single sample.

In the present study, we did not control for the menstrual phase between the patients and controls. In the control group, no data regarding the menstrual phase were obtained. However, in the patient group, an analysis was made and no differences regarding inflammation and stress were detected, except for a slight increase in salivary cortisol values in the premenopausal women compared with the postmenopausal women.

Moreover, in the present study there were no differences in the levels of MMP-8 and MMP-9 between patients and controls. In a study by Yang *et al.* (40), who investigated if stress might modulate the expression of MMPs in plasma, no association was found between depressive symptoms and MMP-8 and MMP-9, but the subjects with a higher level of plasma cortisol had a lower concentration of MMP-2. The authors concluded that the activation of the hypothalamus–pituitary–adrenal cortex can have an impact on the levels of MMPs, although little is known about the relationship.

The present study demonstrated that depressed women on long-term sick leave had more gingivitis, deeper periodontal pockets and higher levels of interleukin-6 in gingival crevicular fluid. An alteration of the immune system in these patients might be interpreted as reflecting consequences of long-term exposure to stress. In such patients, psychological stress could

exacerbate periodontal disorders. It is important that patients with depression are instructed about the relationship between stress and periodontal disease. These patients require individual preventive programmes that are tailored to their needs.

Acknowledgements

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